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INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
James	Russell	Vancouver, CANADA

Additional inventors are being named on the 1 *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Toll-like receptor 2 (TLR-2) haplotypes predict outcome of patients.

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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OR

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 12
☑ Drawing(s) *Number of Sheets* 9
☐ Application Date Sheet. See 37 CFR 1.76
☐ CD(s), Number
☐ Other (specify)

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Enclosures:

1. Provisional application for patent cover sheet
2. Credit card payment for $80.00 filing fee
3. Specifications, 12 pages
4. Drawings, 9 pages

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March 4, 2004

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Re: Provisional Application for "Toll-like receptor 2 (TLR-2) haplotypes predict outcome of patients."

UBC file no: 04-099

Enclosed please find the necessary documents for filing a Provisional Patent Application for the above-identified technology on behalf of The University of British Columbia. Also enclosed is Credit Card payment form PTO-2038 to cover the cost of the $80.00 application fee.

Thank you,

Sincerely,

Elaine A. Weiss, B.Sc, MBA
Technology Transfer Manager

Encl.

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PROVISIONAL APPLICATION COVER SHEET
Additional Page

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INVENTOR(S)/APPLICANT(S)		
Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
Keith	Walley	Vancouver , CANADA

Number 2 of 2

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Title: Toll-like receptor 2 (TLR-2) haplotypes predict outcome of patients.

Inventor: Keith Walley, Vancouver, CANADA
James Russell, Vancouver, CANADA

Abstract:

The invention involves characterization of polymorphisms in the Toll-like receptor 2 (TLR-2) gene that are associated with adverse outcomes in patients. Methodologies for screening haplotypes are described. TLR2 haplotype screening will be useful in identifying patients who would benefit from increased monitoring by healthcare professionals, and/or possible therapeutic intervention, when said patient become subject to inflammation due to systemic inflammation response syndrome (SIRS), bacterial infection, bacteraemia, sepsis, septic shock, organ dysfunction, and trauma.

Background of the Invention:

Systemic inflammatory response syndrome (SIRS) is characterized by increased inflammation (relative to anti-inflammatory processes), increased coagulation (relative to anti-coagulant processes), and decreased fibrinolysis (3, 4, 9, 11, 14, 16). TLR2 is an innate immunity pattern recognition receptor for peptidoglycan, and has an important role in initiating the host immune response to gram-positive bacteria (3). TLR2 binding with peptidoglycan from gram-positive bacteria cell walls initiates intracellular signaling responses that result in the activation of Nuclear Factor Kappa B (NFKß) and the induction of pro-inflammatory cytokines. A polymorphism resulting in a tryptophan being exchanged for an arginine at codon 677 of the TLR2 transcript has been associated with susceptibility to lepromatous leprosy, and with decreased activation of NFKß in response to *Mycobacterium leprae* and decreased serum levels of IL-12 (4, 9). A second polymorphism that results in an Arginine being replaced with a glycine at codon 753 has been associated with decreased TLR2 responsiveness to bacterial peptides from *Borrelia burgdorferi* and *Treponema pallidum*, and with susceptibility to staphylococcal infections in a septic shock population (14).

Using a novel haplotype-based analysis, the inventors have identified a single nucleotide polymorphism (SNP) in the TLR2 gene that identifies a family of TLR2 haplotypes (clade) that are associated with statistically significant differences in important measures of clinical outcome such as survival and organ dysfunction. The inventors describe herein the novel observation that the –16933A/A polymorphism is a marker of increased risk of gram-positive infection, bacteremia and sepsis in critically ill patients. The present invention describes a better strategy of predicting patients who are at a greater risk of an adverse outcome,

thus enabling earlier intervention and facilitating patient-tailored therapy based on genotype.

Summary of the Invention:

The present invention is concerned with single nucleotide polymorphisms (SNPs), which form haplotypes within the toll-like receptor 2 gene (TLR2), which are predictive of patient outcome should that patient experience inflammation. Examples of inflammation experienced by patients include, but are not limited to, systemic inflammation response syndrome (SIRS), bacterial infection, bacteraemia, sepsis, septic shock, organ dysfunction, and trauma. This invention is novel, as the respective grouping of haplotypes described in the invention predict risk of gram-positive infection, bacteremia and sepsis much more accurately patient outcome much more accurately than previously identified TLR2 polymorphisms.

In one aspect, the present invention provides the methodology required to screen patients in order to determine those at risk of an adverse outcome following inflammation. Genetic material is collected from the patient, most commonly by isolating leukocytes from the blood, but alternatively through a variety of biopsy methods, in order that the haplotype of the TLR2 gene can be ascertained. Determination of the haplotype from the genetic material can be done through a variety of methods commonly described in the art, including, but not limited to sequencing, restriction fragment length polymorphism (RFLP) analysis, hybridization, oligonucleotide ligation assay, ligation rolling circle amplification, allele specific PCR, and single base-pair extension assays. Sequence data from any of the above mentioned assays could be stored in a database for future retrieval and haplotype analysis.

In another aspect, the present invention provides a test to predict the presence of gram positive bacteria in a patient hospitalized for various reasons, including sepsis, glomerulonephritis, bowel infection, pneumonia, HIV/AIDS, cystic fibrosis diabetes mellitus, chronic renal failure, peritonitis, bronchiectasis, chronic obstructive lung disease, chronic bronchitis, emphysema, asthma, febrile neutropenia, fever of unknown origin, meningitis, septic arthritis, urinary tract infection, necrotizing fasciitis, recurrent or suspected Staphylococcus aureus infection, recurrent or suspected Group A Streptococcus infection, recurrent or suspected enterococcus infection, opportunistic infections, major surgery or dialysis, immunocompromized, or other medical and surgical conditions associated with increased risk of infection.

In another aspect of the invention, those patients at highest risk of gram positive infection are the infirm, elderly, and those individuals requiring

hospitalization for a variety of reasons including sepsis, glomerulonephritis, bowel infection, pneumonia, HIV/AIDS, cystic fibrosis diabetes mellitus, chronic renal failure, peritonitis, bronchiectasis, chronic obstructive lung disease, chronic bronchitis, emphysema, asthma, febrile neutropenia, fever of unknown origin, meningitis, septic arthritis, urinary tract infection, necrotizing fasciitis, recurrent or suspected Staphylococcus aureus infection, recurrent or suspected Group A Streptococcus infection, recurrent or suspected enterococcus infection, opportunistic infections, major surgery or dialysis, immunocompromized, or other medical and surgical conditions associated with increased risk of infection. These at risk individuals could be screened for the TLR2 haplotypes associated with increased risk of gram positive infection such that those individuals can benefit from increased monitoring, and possibly earlier prophylactic treatments, in order to reduce the incidence of gram positive infections, bacteremia and sepsis.

In another aspect of the invention, patients suffering from gram positive infection, bacteremia or sepsis could be screened for the TLR2 haplotypes associated with increased risk of gram positive infection, bacteremia or sepsis such that those individuals can benefit from increased monitoring, and possible prophylactic treatments begun in order to reduce the incidence of gram positive infections, bacteremia and sepsis.

In another aspect of the invention, the invention provides the methodology required to determine patient outcome following collection of genetic material and haplotype determination by analysing the TLR2 gene, whereby the specific TLR2 SNPs that form the respective haplotypes are located in the sequence described in SEQ ID NO:1.

In another aspect, the invention further provides the methodology required to determine patient outcome following collection of genetic material and haplotype determination by analysing the TLR2 gene, whereby 4 major haplotype clades could be defined by identifying the SNP at position -16933 of TLR2, relative to the transcriptional start site.

In another aspect, the invention further provides the methodology required to determine patient outcome following collection of genetic material by analysing the TLR2 gene at position –16933, relative to the transcriptional start site, whereby those individuals who have the minor adenine (A) allele at position -16933 display an adverse outcome. This adverse outcome is due to decreased survival arising from infection causing or resulting from organ dysfunction, SIRS, sepsis, septic shock, bacterial infection, bacteraemia, or trauma.

The converse of the above paragraph is also true, as the same methodology required to determine patient outcome following collection of genetic material and analysing the TLR2 gene at position –16933, relative to the transcriptional

start site, whereby those individuals who do not have the minor adenine (A) allele at position -16933 do not display an adverse outcome such as gram positive infection due to or resulting from organ dysfunction, SIRS, sepsis, septic shock, bacterial infection, bacteraemia, or trauma.

The sequence positions referred to in this invention and detailed in SEQ ID NO:1 refer to the sense strand of the TLR2 gene. It will be obvious to a person skilled in the art that analysis could be conducted on the anti-sense strand to determine patient outcome.

The invention further provides for kits useful in carrying out the methods of the invention.

Brief Description of the Drawings:

Figure 1. Haplotype structure of the TLR2 gene.
Columns are polymorphic sites. Rows are haplotypes of TLR2 ordered by phylogenetic relationship (Figure 2). Yellow boxes are minor alleles and blue boxes are major alleles. T-16933A was chosen as a "tag" SNP to distinguish between the 2 major haplotype clades of the TLR2 gene.

Figure 2. TLR2 haplotypes evolutionary relationships.
Haplotypes were sorted into 2 clades according to evolutionary tree structure. Clades are labelled by the alleles at -16933. Tree branch distance is % difference between haplotype sequences. (Scale bar = 2% sequence difference).

Figure 3. 28 day mortality rates by TLR2 clade.
(A) There was no difference in 28 day mortality rates between TLR2 clades.
(B) Kaplan-Meier analysis of censored mortality rates indicates there was no difference in mortality rates between TLR2 clades at any point of the 28 day study period.

Detailed Description of the Invention:

Definitions

Allele — One of the variant forms of a gene at a particular locus, or location, on a chromosome. Different alleles produce variation in inherited characteristics such as hair color or blood type. In an individual, one form of the allele (the dominant or major one) may be expressed more than another form (the recessive or minor one).

Clade — A group of haplotypes that are closely related phylogenetically. For example, if haplotypes are displayed on a phylogentic (evolutionary) tree a clade includes all haplotypes contained within the same branch.

Genetic Material — Genetic material refers to nucleic acids, whether deoxyribonucleic acid or ribonucleic acid, isolated from cells acquired from tissue or organisms.

Genotype — Genotype refers to the genetic makeup of an organism.

Haplotype — The set of genes, comprised of one allele of each gene, which make up the genotype.

Phenotype — Phenotype refers to the observable characteristics of an organism produced by the organism's genotype interacting with the environment.

Single Nucleotide Polymorphism (SNP) — A SNP is a place in the genetic code where DNA differs from one person to the next by a single nucleotide base pair. These slight genetic variations between human beings may predispose some people to disease and explain why some respond better to certain drugs.

Methods

Patient Cohort — All patients admitted to the Intensive Care Unit (ICU) of St. Paul's Hospital were screened for inclusion. This ICU is a mixed medical – surgical ICU in a tertiary care, university-affiliated teaching hospital of the University of British Columbia. We only report the results for the Caucasian patients (78.6% of all admissions) who were successfully genotyped (n=223) in order to decrease the potential confounding influence of population admixture secondary to ethnic diversity, on associations between genotype and phenotype.

Clinical Phenotype — Our primary outcome variable was survival to hospital discharge. Secondary outcome variables were days alive and free of cardiovascular, respiratory, renal, hepatic, hematologic, and neurologic organ system failure as well as days alive and free of SIRS (Systemic Inflammatory Response Syndrome), occurrence of sepsis, and occurrence of septic shock. SIRS was considered present and the patients included in the study when patients met at least two of four SIRS criteria. The SIRS criteria were 1) fever (>38 °C) or hypothermia (<35.5 °C), 2) tachycardia (>100 beats/min in the absence of beta blockers, 3) tachypnea (>20 breaths/min) or need for mechanical ventilation, and 4) leukocytosis (total leukocyte count > 11,000/μL(1). Patients were included in this cohort on the calendar day on which the SIRS criteria were met.

Patients' baseline demographics that were recorded included age, gender, whether medical or surgical diagnosis for admission (according to APACHE III diagnostic codes (11), and admission APACHE II score. The following additional data were recorded for each 24 hour period (8 am to 8 am) for 28 days to evaluate organ dysfunction, SIRS, sepsis, and septic shock.

Clinically significant organ dysfunction for each organ system was defined as present during a 24 hour period if there was evidence of at least moderate organ dysfunction using the Brussels criteria (Table 1) (15). Because data were not always available during each 24 hour period for each organ dysfunction variable, we used the "carry forward" assumption as defined previously (2). Briefly, for any 24 hour period in which there was no measurement of a variable, we carried forward the "present" or "absent" criteria from the previous 24 hour period. If any variable was never measured, it was assumed to be normal.

To further evaluate cardiovascular, respiratory, and renal function we also recorded, during each 24 hour period, vasopressor support, mechanical ventilation, and renal support, respectively. Vasopressor use was defined as dopamine > 5 μg/kg/min or any dose of norepinephrine, epinephrine, vasopressin, or phenylephrine. Mechanical ventilation was defined as need for intubation and positive airway pressure (i.e. T- piece and mask ventilation were not considered ventilation). Renal support was defined as hemodialysis, peritoneal dialysis, or any continuous renal support mode (e.g. continuous veno-venous hemodialysis).

To assess duration of organ dysfunction and to correct organ dysfunction scoring for deaths in the 28 day observation period, we calculated days alive and free of organ dysfunction (DAF) as previously reported (2). Briefly, during each 24 hour period for each variable, DAF was scored as 1 if the patient was alive and free of organ dysfunction (normal or mild organ dysfunction, Table 1). DAF was scored as 0 if the patient had organ dysfunction (moderate, severe, or extreme) or was not alive during that 24 hour period. Each of the 28 days after ICU admission was scored in each patient in this fashion. Thus, the lowest score possible for each variable was zero and the highest score possible was 28. A low score is indicative of more organ dysfunction as there would be fewer days alive and free of organ dysfunction.

Similarly, we calculated days alive and free of SIRS (DAF SIRS). Each of the four SIRS criteria were recorded as present or absent during each 24 hour period. Presence of SIRS during each 24 hour period was defined by having at least 2 of the 4 SIRS criteria. Sepsis was defined as present during a 24 hour period by having at least two of four SIRS criteria and having a known or suspected infection during the 24 hour period. Cultures that were judged to be positive

due to contamination or colonization were excluded. Septic shock was defined as presence of sepsis plus presence of hypotension (systolic blood pressure < 90 mmHg or need for vasopressor agents) during the same 24 hour period.

Microbiology – Microbiological cultures were taken for any patients who were suspected of having an infection. As this is a cohort of critically ill patients with SIRS, most patients had cultures taken. Positive cultures that were suspected of having been contaminated or colonized were excluded. Positive cultures that were deemed to clinically be clinically irrelevant were also excluded. Cultures were categorized as gram positive, gram negative, fungal or other. The sources of the cultures were respiratory, gastrointestinal, skin, soft tissues or wounds, genitourinary, or endovascular.

Haplotypes and Selection of htSNPs — Using unphased Caucasian genotypic data from the Coriell registry (from www.innateimmunity.net/IIPGA/IIPGASNPs), we inferred haplotypes of TLR2 using PHASE software (Figure 1) (17). We then used MEGA 2 software (12) to infer a phylogenetic tree to identify major haplotype clades (Figure 2). Haplotypes were sorted into clades according to this phylogenetic tree and this haplotype structure was inspected to choose htSNPs (5, 8). We chose an htSNP that identified the 2 major haplotype clades of TLR2 in Caucasians (rs4696480, position –16933 relative to the transcription start site of the TLR2 gene. This SNP was then genotyped in our patient cohort to define major clades.

Blood collection and processing – The buffy coat was extracted from whole blood and samples transferred into 1.5 ml cryotubes and stored at –80°F. DNA was extracted from the buffy coat using the Qiagen DNA Blood Mini Kit. The genotypic analysis was performed in a blinded fashion, without clinical information.

Genotyping — Patients' genotypes at T-16933A were determined by real time polymerase chain reaction (PCR) assay using specific fluorescence-labeled hybridization probes in the ABI Prism 7900 HT Sequence Detection System (Applied Biosystems, Inc.) as described by Livak (13). Briefly, the ABI Prism 7900HT uses a 5′ Nuclease Assay in which an allele-specific probe labeled with a fluorogenic reporter dye and a fluorogenic quencher is included in the PCR reaction. The probe is cleaved by the 5′ nuclease activity of Taw DNA polymerase if the probe target is being amplified, freeing the reporter dye and causing an increase in specific fluorescence intensity. Mismatched probes are not cleaved efficiently and thus do not contribute appreciably to the final fluorescent signal. An increase in a specific dye fluorescence indicates homozygosity for the dye-specific allele. An increase in both signals indicated heterozygosity. DNA from lymphocyte cell lines obtained from the Coriell Cell Repository was used to ensure the accuracy of the genotyping. The genotype of

these cell lines as determined using the ABI Prism 7900HT Sequence Detection system was compared to the genotype of the same cell lines determined by direct sequencing, given at *www.innateimmunity.net/IIPGA/IIPGASNPs*.

Statistical Analysis – A cohort study design was used. Rates of dichotomous outcomes (28-day mortality, sepsis and shock at onset of SIRS) were compared between T-16933A genotypes using a chi-squared test. Differences in continuous outcome variables between genotypes were tested using ANOVA. 28-day mortality was further compared between genotypes while adjusting for other confounders (age, sec, and medical vs. surgical diagnosis) using a Cox regression analysis in addition to a Kaplan-Meier analysis. Genotype relative risk was calculated. Genotype distributions were tested for Hardy-Weinberg equilibrium (7). We report the mean and 95% confidence intervals. Statistical significance was set at $p<0.05$. The data was analyzed using SPSS 11.5 for Windows and SigmaStat 3.0 software (SPSS Inc., Chicago, IL, 2003).

Discussion

We tested the hypothesis that haplotype clades of the TLR2 gene would be associated with rates and types of infection in a population of critically ill patients (n=252). In order to narrow down the choice of possible candidate SNPs in TLR2 we tested the hypothesis that haplotype clades (evolutionarily related families of haplotypes) are associated with clinical outcome in a critically ill Caucasian cohort (n=223) of consecutively admitted patients to a tertiary care Intensive Care Unit.

Using Cladistic analysis of TLR2 gene haplotypes deduced from data available on public databases, we determined a minimum set of single nucleotide polymorphisms that defined all major haplotype clades of the TLR2 gene. A T-to-A polymorphism at position -16933 relative to the start transcription site of the TLR2 gene (rs4696480) marked a haplotype clade that encompassed 47% of the haplotypes occurring within the TLR2 gene. We found that the -16933 A/A genotype was associated with increased incidence of sepsis upon admission to the ICU. 79% of patients with the -16933 A/A genotype had sepsis upon admission to the ICU, while only 60% of patients with the -16933 A/T or T/T genotypes had sepsis upon admission ($p<0.007$). The -16933 A/A genotype was also associated with an increased prevalence of gram positive cultures. The distribution of the T-16933A genotype was significantly altered in patients who had a culture positive for gram positive bacteria. 40% of patients with a gram positive culture were homozygous for the A allele at position -16933, while only 22% of patients who did not have a gram positive culture were homozygous for the A allele at position -16933 of the TLR2 gene ($p<0.02$). There was furthermore a specific association of the A/A and A/T genotypes with positive blood cultures. 90% of patients with positive endovascular cultures had the A/A

or A/T genotypes at position -16933, while only 74% of patients who had a positive culture from another source (respiratory, gastrointestinal, skin or soft tissues, or genitourinary) had the A/A or A/T genotype ($p<0.05$). We conclude that the -16933 A/A genotype is a predictor of increased risk of gram positive infection, gram positive bacteremia, and sepsis in critically ill patients.

Examples:

252 consecutive critically ill patients admitted to the ICU of St. Paul's Hospital were screened for inclusion. Of these, 223 Caucasian patients were successfully genotyped and make up the cohort of this study.

Example 1

Haplotype clade deduction

We were able to infer haplotypes from complete sequencing of TLR2 for 23 Caucasians in the Coriell Cell Repository using PHASE software, and identified two major haplotype clades using MEGA2 software (Figures 1 and 2). These haplotype clades could be resolved by genotyping the "haplotype tag" SNP ()htSNP) T-16933A (rs4696480) in our cohort of critically ill patients. The minor T allele of this polymorphism marks a clade which encompasses 47% of the haplotypes found in the TLR2 gene (Figure 1). The genotype frequencies of this polymorphism were similar to frequencies deduced from other available Caucasian data from (www.innateimmunity.net/IIPGA/IIPGASNPs), and were in Hardy-Weinberg equilibrium (Table 2). We chose a haplotype clade-based approach to test the association of polymorphisms to outcome as we have found that it reduces the potential amount of genotyping almost 30-fold (28 to 1 genotyped SNP) with little loss of information, as there is less difference between haplotypes within a clade than between haplotypes from different clades (Table 3) (10). The T-16933A SNP is at a major branch point of the TLR2 haplotype phylogenetic tree and thus splits the haplotypes of TLR2 into 2 clades of approximately equal size (Figure 2). Haplotype 6 falls into the clade defined by the –16933A allele although it does not appear to be closely related to other haplotypes within the clade defined by the –16933A allele. This may be the result of a reverse mutation in this haplotye from the –16933T allele back to the –16933A allele at some point in history.

Example 2

Haplotype patient outcome

For the 223 successfully genotyped individuals of the cohort of Caucasian patients who had at least 2 of 4 SIRS criteria, no genotype of TLR2 T-16933A

was significantly associated with a difference in age, gender, medical vs. surgical diagnosis for admission, or severity of illness at time of admission (as estimated by the APACHE II score) (Table 4).

The T-16933A polymorphism was not associated with our primary outcome variable, 28-day mortality 9Figure 3a). Patients of all genotypes of the T-16933A SNP had similar rates of 28-day mortality over the entire 28-day observation period (Figure 3b), and T-16933A genotype was not an independent predictor of mortality in a Cox regression model.

In our cohort of critically ill patients the TLR2 –16933A/A genotype was associated with an increased rate of sepsis upon admission to the study. 79% of patients who were homozygous for the –16933A allele had sepsis on day one of the observation period, compared to only 60% of patients who were heterozygous or homozygous for –16933T allele (Fisher's exact, $p<0.007$). Patients with the –16933A/A genotype had a relative risk of 1.3 of having sepsis on day one (95% CI = 1.1-1.6).

Interestingly, TLR2 T-16933A genotype was found to be predictive of the occurrence of gram positive cultures in our cohort of critically ill patients. 40% of patients with a gram positive culture were homozygous for the A allele at position –16933, while only 22% of patients who did not have a gram positive culture were homozygous for the A allele at position –16933 of the TLR2 gene (Fisher's exact, $p<0.02$). patients who were homozygous for the –16933A allele were twice as likely to have a gram positive culture as those patients who were not homo9zygous for the –16933A allele (RR=2.0, 95% CI=1.1-3.4). There was furthermore, a specific association of the A/A and A/T genotypes with positive blood cultures. 90% of patients with positive endovascular cultures had the A/A or A/T genotypes at position –16933, while only 74% of patients who had a positive culture from another source (respiratory, gastrointestinal, skin or soft tissues, or genitourinary) had the A/A or A/T genotype (Fisher's exact, $p<0.05$).

The –16933A/A genotype was not significantly associated with an increased risk of septic shock on day one of the study, nor with an increased risk of septic shock at any time over the course of the 28-day observation period. The T-16933A polymorphism was not associated with a difference in any of our measures of organ dysfunction or organ support.

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TABLE 1
Brussels Organ Dysfunction Scoring System

ORGANS	Free of Organ Dysfunction		Clinically Significant Organ Dysfunction		
	Normal	Mild	Moderate	Severe	Extreme
Cardiovascular Systolic BP (mmHg)	>90	≤90 Responsive to fluid	≤90 Unresponsive to fluid	≤90 plus pH ≤7.3	≤90 plus pH ≤7.2
Pulmonary P_aO_2/F_IO_2 (mmHg)	>400	400-301	300-201 Acute lung injury	200-101 ARDS	≤100 Severe ARDS
Renal Creatinine (mg/dL)	<1.5	1.5-1.9	2.0-3.4	3.5-4.9	≥5.0
Hepatic Bilirubin (mg/dL)	<1.2	1.2-1.9	2.0-5.9	6.0-11.9	≥12
Hematologic Platelets ($x10^5/mm^3$)	>120	120-81	80-51	50-21	≤20
Neurologic (Glascow Score)	15	14-13	12-10	9-6	≤5
Round Table Conference on Clinical Trials for the Treatment of Sepsis Brussels, March 12-14, 1994 (15)					

TABLE 2
Genotype and Allele Frequencies of TLR2 haplotype tag SNP T-16933 A in a Cohort of Critically Ill Adults who had SIRS

	Genotype Frequencies			Allele Frequencies		p*
	AA	TA	TT	A	T	
T-16933A	25%	51%	24%	51%	49%	0.796

* exact test of Guo and Thompson to test for Hardy-Weinberg equilibrium (6)

TABLE 3 Use of Haplotype-Based Analysis in Association Studies

Gene	Size of sequenced DNA (bp)	No. of SNPs	No. of Haps	No. of clades	No. of htSNPs	No. of haps per clade	Ave % diff. btw haplotypes w/in a clade	% diff. btw clades
TLR2	33165	28	10	2	1	5	9.3%	13.1%

TABLE 4. Baseline characteristics and mortality of 252 critically ill patients who had SIRS

Genotype	Mean Age	Gender (% Male)	Diagnosis for admission (% Surgical)	Mean APACHE II	28-day Mortality
AA	61	65%	39%	21	34%
AT	59	68%	24%	19	35%
TT	58	62%	25%	19	33%
p	NS	NS	0.03	NS	NS

Figure 1. Haplotype structure of the TLR2 gene in Caucasians.

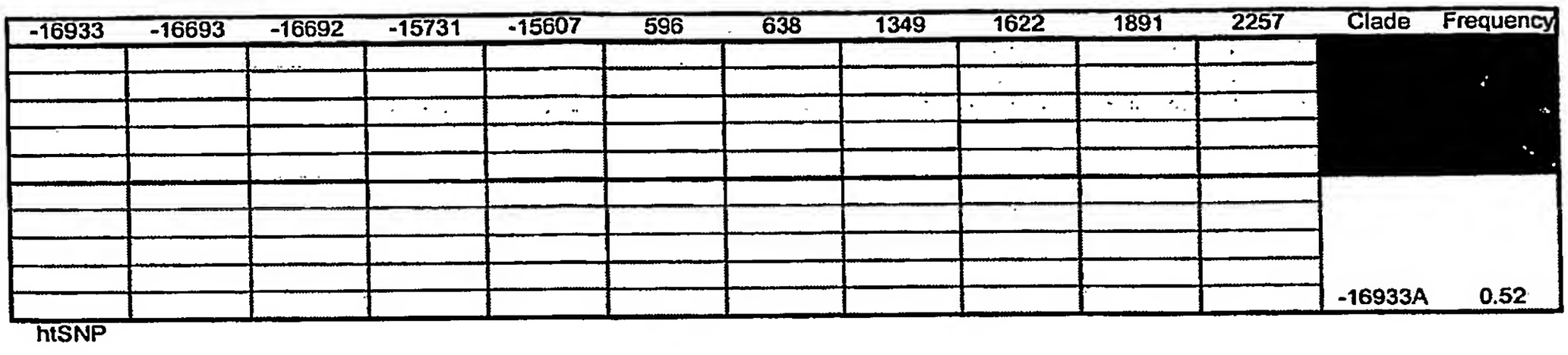


Figure 2. Unrooted phylogenetic tree of TLR2 haplotype clades.

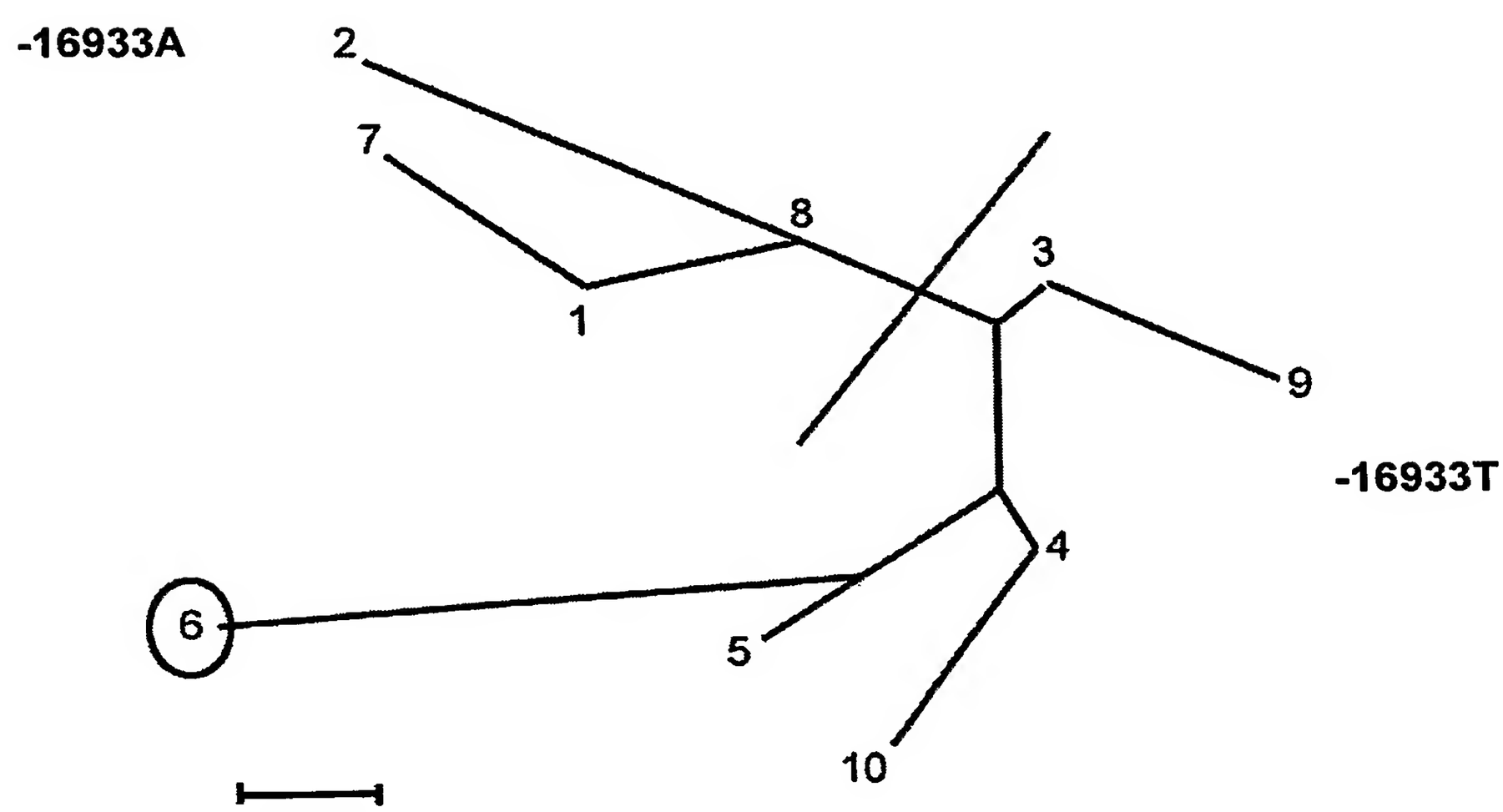


Figure 3a. 28 day Mortality in critically ill patients with SIRS by TLR2 T-16933A genotype.

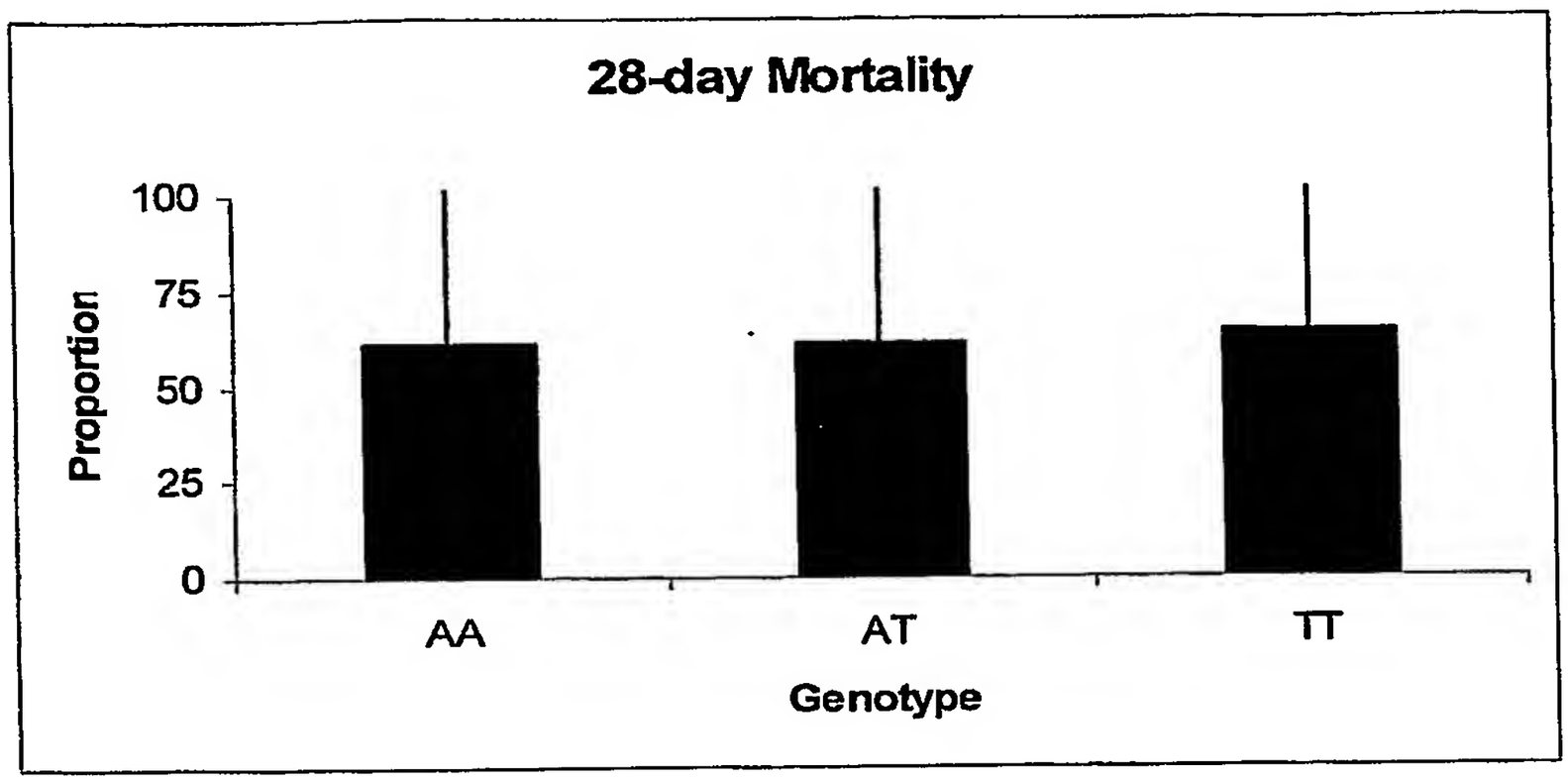
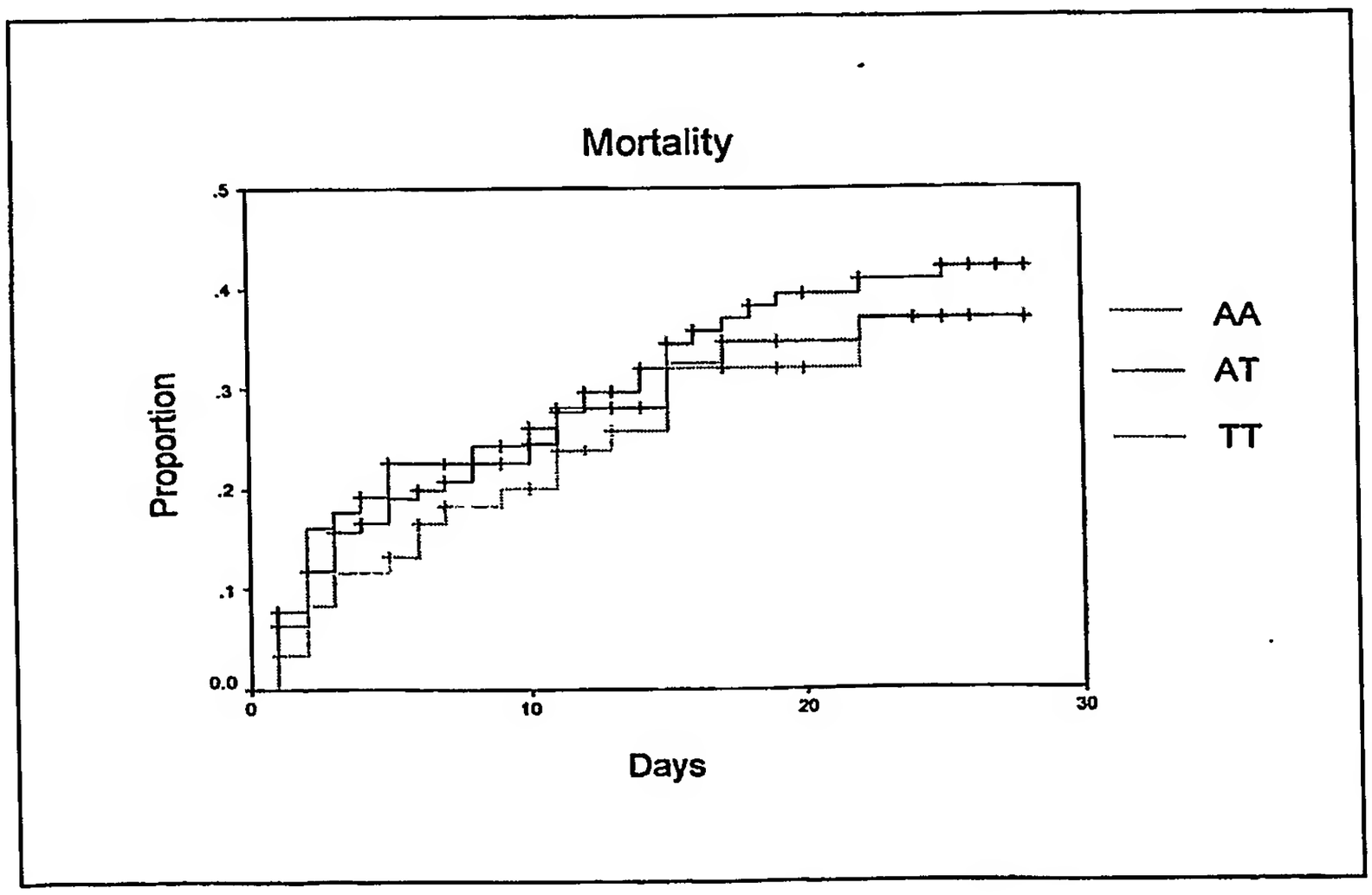


Figure 3b. Kaplan-Meier Survival analysis of critically ill patients by TLR2 T-16933A genotype.



SEQ ID. NO. 1

T-16933A
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GTTCTGGAGTCTGGGAAGTCCAAGATTGAAGGGCTGCATCTGG[T/A]GAGGGTCAT
CTGGCTACATTATAACATGATGGAAAGCATCACATGGTGAGAGAGAGCAAGAGAGG
ACAGAACTTACTTTTATAACAAACTCATTCTCACA